Identification of Potential AcrAB-TolC Efflux Pump Inhibitors in *Escherichia coli* using an Ethidium Bromide Method

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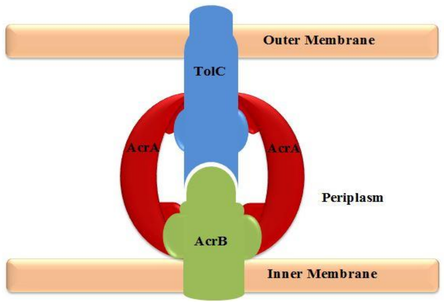
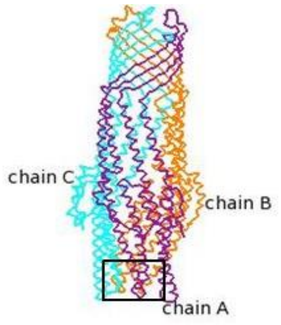
Research objective: To obstruct a bacterial efflux pump though physical binding of small molecule inhibitory compounds in order to combat substrate expulsion.

Introduction

- Antibiotic resistance in various bacteria
- Some resistance can be attributed to overexpression of efflux pumps
- Trimeric efflux complex uses proton motive force to move substrates through the periplasm, towards the extracellular space
- TolC protomer contains single 100 Å pore spanning entire subunit
- Evidence of a variety assembly mechanisms contributing to the formation of AcrAB-TolC pump



AcrAB-TolC Efflux Pump Complex

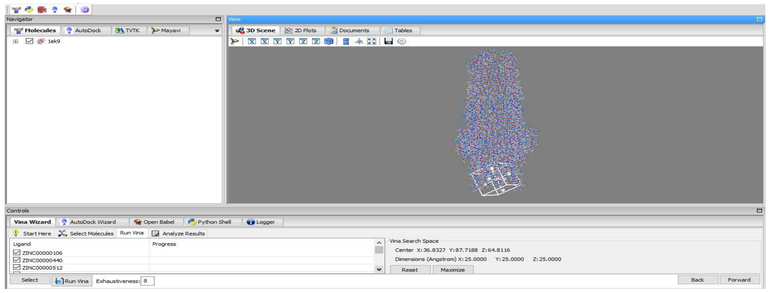


TolC Target Site

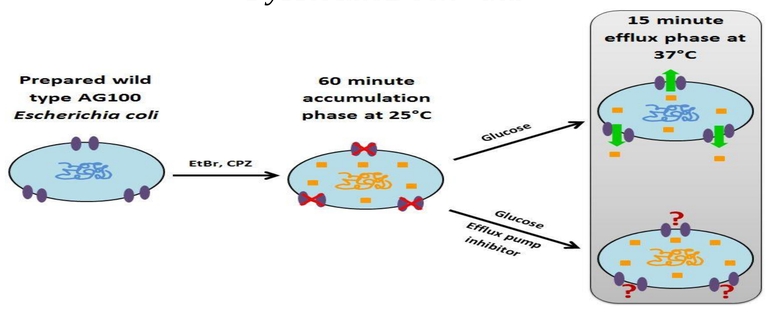
Materials and Methods

Virtual Screening:

- Target site: TolC subunit (protoplasmic pore) of AcrAB-TolC efflux complex
- Three source catalogs selected for screening (54,780 total compounds)
- PyRx AutoDock Vina used to screen for low predicted binding energies
  - Electrostatic and noncovalent interactions
  - Steric parameters



PyRx AutoDock Vina



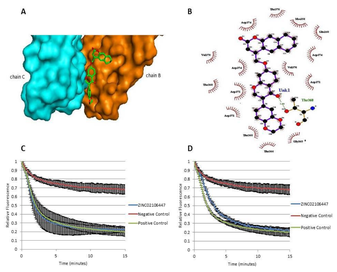
In Vivo Efflux Assay using Ethidium Bromide (EtBr) as a Fluorescent Tag

Results

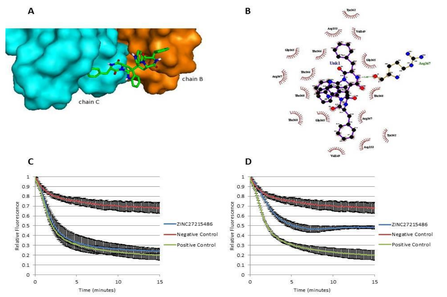
- No significant difference in efflux levels in systems without prolonged compound incubation
- Efflux pump activity decreased in three out of five total systems with prolonged compound incubation

Zinc ID	Predicted Binding Energy (kcal/mol)	Chemical Structure
ZINC02106447	-10.8	
ZINC27215486	-10.3	
ZINC61971127	-10.2	
ZINC53683906 (Sucrose)	-6.0	

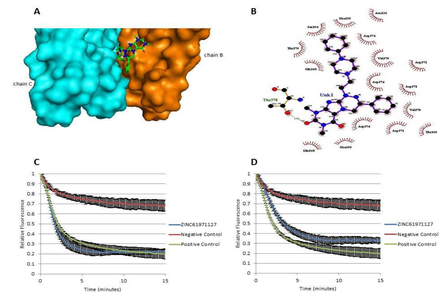
Predicted Binding Energies



ZINC02106447 In Vivo Efflux Activity



ZINC27215486 In Vivo Efflux Activity



ZINC61971127 In Vivo Efflux Activity

Discussion

- Lead-compounds introduced immediately before efflux may:
  - Not be able to make it to the intended site
  - Be easily metabolized
  - Be simply effluxed
- Efflux pump inhibition under prolonged compound incubation for select small molecules may:
  - Cause misfolding and conformational changes of the TolC protomer
  - Prevent assembly with the rest of the efflux complex

Future Research

- Immunostaining to determine AcrAB-TolC protein concentrations *in vivo* with and without prolonged compound incubation
- Gel electrophoresis to test for protein:protein interactions among AcrA, AcrB, and TolC in the presence and absence of the lead-compounds
- Screening of ZINC27215486 analogs to test compounds with similar effective binding patterns